V02

Bacterial genome DNA extraction kit

Cat: D1600
Package: 50T/100T
Storage: RT, Valid for 1 year (RNase A is shipped as an attachment and stored at -20℃).

Product composition:

Kit composition	50T	100T	Storage
RNase A	100μL×2	100μL×4	-20℃
Protease K	1mL	1mL×2	-20℃
Solution A	15mL	30mL	RT
Solution B	15mL	30mL	RT
Bleaching solution	15mL	15mL×2	RT
Eluent	10mL	20mL	RT
Adsorption column	50	100	
Collecting tube	50	100	
Specification	1	1	

Notes:

1. Please add anhydrous ethanol to the bleach solution before use, and add the volume according to the label on the bottle (each bottle needs to add 45mL anhydrous ethanol separately).
2. All centrifugal steps are centrifuged at room temperature using a table centrifuge.
3. This kit is only applicable to Gram-negative bacteria and some Gram-positive bacteria. If it is difficult to extract the genome of Gram-positive bacteria, you need to bring some reagents or purchase the D1650-Gram-positive bacteria genome DNA extraction Kit.

Product description:

This kit uses a centrifugal adsorption column that can specifically bind DNA and a unique buffer system to extract bacterial genomic DNA. The silicon matrix material used in the centrifugal adsorption column is our company's unique new material, which can efficiently and specifically adsorb DNA, and can maximize the removal of foreign proteins and other organic compounds in the cell. The extracted genomic DNA fragments are large, high purity, stable and reliable. Genomic DNA extracted using this kit can be used for a variety of routine operations, including enzyme digestion, PCR, library construction, Southern hybridization and other experiments.

Operation steps:

1. Take 1-5mL of bacterial culture medium, centrifuge at 12000rpm for 1min, and remove the supernatant as far as possible.
2. Add 250μL solution A to the bacteria, shake or blow with A pipette to fully suspend the bacteria, add 4μL RNase A to the suspension, shake for 15s, and leave for 5min at room temperature.

[Note]

If it is Gram-positive bacteria, the lysozyme solution can be added before the second step for wall breaking treatment. The lysozyme needs to be prepared by itself, the specific method is: Add 500μL 70% ethanol to the bacteria, ice bath for 20min, centrifuge at 12000rpm for 1min, discard the supernatant, precipitate the bacteria and add 70μL lysozyme solution (50-100mg/mL), and treat at 37℃ for 30-60min.

3. Add 20μL protease K (10mg/mL) into the tube, mix it well, and place it at 70℃ for 10min. At this time, the bacterial solution is clear and viscous.
4. Add 220μL solution B into the tube, shake for 15s, place at 70℃ for 10min, and centrifuge instantaneously to remove the water beads on the inner wall of the tube cover.
5. Add 220μL of anhydrous ethanol into the tube, mix it well, shake for 15s, the solution becomes clear, and flocculation precipitation may occur at this time, which does not affect the extraction of DNA. Both the solution and flocculation precipitation can be added to the adsorption column and left for 2min.
6. Centrifuge at 12000rpm for 2min, discard the waste liquid, and put the adsorption column into the collection

Tel: 400-968-6088 https://www.solarbio.com E-mail: sales-china@solarbio.com
Address: No. 85, Liandong U Valley, Middle Zone, 101102, Tongzhou Dist, Beijing, China

tube.

7. Add 600μL bleach solution to the adsorption column (check whether anhydrous ethanol has been added before use). Centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column into the collection tube.
8. Add 600μL bleach solution to the adsorption column. Centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column into the collection tube.
9. Centrifuge at 12000rpm for 2min and place the adsorption column open at room temperature or 50℃ for several minutes to remove the residual bleach solution in the adsorption column; otherwise, the ethanol in the bleach solution will affect subsequent experiments, such as enzyme digestion and PCR.
10. Put the adsorption column into a clean centrifuge tube, add 50-200μL eluent preheated in a water bath at 65℃ to the center of the adsorption film, place at room temperature for 5min, and centrifuge at 12000rpm for 1min.
11. The eluent obtained by centrifugation was added to the adsorption column, placed at room temperature for 2min, and centrifuged at 12000rpm for 2min to obtain high quality bacterial genomic DNA.

Notes:

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be small and the extracted amount will decrease.
2. If the solution in the kit is precipitated, it can be re-dissolved in a 65℃ water bath before use, without affecting the extraction effect.
3. If the column is blocked in the centrifugal step of the experiment, the centrifugal time can be appropriately extended.
4. The volume of eluting buffer is better than 50μL, too small volume will affect the recovery efficiency; The pH value of the eluent also has an impact on the elution efficiency. If water is needed to make the eluent, ensure that its pH value is around 8.0 (NaOH can adjust the pH value of water to this range). A pH value lower than 7.0 will reduce the elution efficiency. DNA products should be stored at -20 ° C to prevent DNA degradation.
5. DNA concentration and purity detection: The size of the obtained genomic DNA fragments is related to the sample storage time, shear force during operation and other factors. The concentration and purity of the recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. DNA should have a significant absorption peak at OD260, where an OD260 value of 1.0 corresponds to approximately 50μg/mL double-stranded DNA and 40μg/mL single-stranded DNA. The OD260/0D280 ratio should be 1.7-1.9, if the elution buffer is not used, but deionized water is used, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but does not indicate low purity.

Related products:

D1010 6×DNA Loading Buffer
T1060 50×TAE Buffer
T1050 5×TBE Buffer
G8142 GoldView Type II nucleic Acid Stain (5000×)
D1100 Small plasmid extraction kit
D1600 Bacterial genome DNA extraction kit
D1700 Animal tissue/cell genome DNA extraction kit
D1800 Whole blood genome DNA extraction kit

Related literature:

[1] Jirong Lan,Yan Sun,Li Guo,et al. A novel method to recover ammonia, manganese and sμLfate from electrolytic manganese residues by bio-leaching. Journal of Cleaner Production. June 2019;499-507. (IF 5.651)

Note: For more information about this product, please refer to Solarbio website.